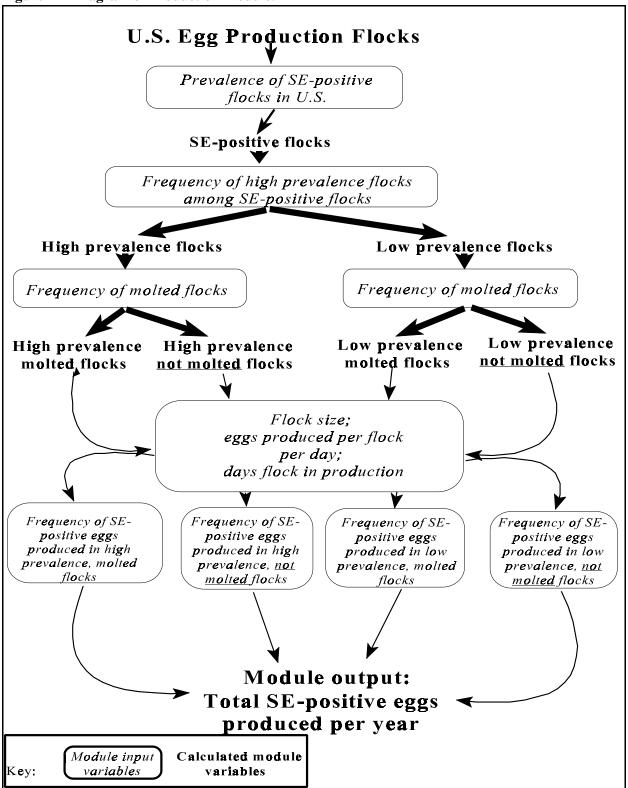


## A. Summary of Production Module

The purpose of the production module is to simulate the annual production of SE-positive eggs in the USA. Through the incorporation of epidemiologically relevant variables, this production module may also be used to simulate the effects of specific mitigation strategies on the annual production of SE-positive eggs.

The production module is the first stage of a farm-to-table quantitative risk assessment of the exposure of the human population to SE-positive eggs and the adverse medical outcomes which may occur as a result of this exposure. Once the total number of egg-producing flocks is specified, this module completes probability calculations to determine the number of SE-positive flocks and the number of SE- negative flocks within the total number of egg-producing flocks. SE-positive flocks are further differentiated into 'low prevalence SE-positive flocks' and into 'high prevalence SE-positive flocks'. Low prevalence SE-positive flocks are defined as flocks which produce SE-positive eggs, but produce SE-positive eggs at a very low rate - e.g. 1 SEpositive egg per 17,000 eggs laid by the low prevalence SE-positive flock. High prevalence SEpositive flocks are defined as flocks which produce SE-positive eggs, and produce SE-positive eggs at a higher rate - e.g. 1 SE-positive egg per 1400 eggs laid by the high prevalence SEpositive flock. For a given number of SE-positive flocks, the module then determines the number of these SE-positive flocks that would be expected to be 'high prevalence SE-positive flocks' and the number of these SE-positive flocks that would be expected to be 'low prevalence SE-positive flocks'. Some egg laying hens also go through a molting process during which they are rejuvenated to lay eggs for a longer period of time. Molting is associated with an increased rate of SE-positive eggs within SE-positive flocks. In recognition of this fact, the production module also calculates the number of SE-positive flocks that are molted. Consequently, the model calculates the number of SE-positive flocks in each of the following categories:

Figure A-1 Diagram of Production Module.



- a) high prevalence SE-positive flock, molted,
- b) high prevalence SE-positive flock, not molted,
- c) low prevalence SE-positive flock, molted, and
- d) low prevalence SE-positive flock, not molted.

The production module calculates the number of SE-positive eggs produced annually for each of these categories. The sum of these calculations is the total number of SE-positive eggs produced annually by the egg industry, and this sum is the primary output of the production module.

As a final step in the production module, the number of SE-positive eggs marketed to shell egg processing & distribution and the number of SE-positive eggs marketed to liquid egg processing is estimated. This step determines the total number of SE-positive eggs which the Shell Egg Processing & Distribution Module will handle and the total number of SE-positive eggs which the Liquid Egg Processing Module will handle.

## **B.** Inputs to Production Module

The number of flocks modeled in the production module is constant and is equal to 5028 flocks, a figure adapted from the 1992 U.S. Agriculture Census data. To account for the variability in egg production for different sized flocks, the total number of flocks are stratified by size according to 1992 U.S. Agriculture Census data (Table A-1).

For the purposes of this model, a flock is defined as a group of hens of similar age which are housed together. The U.S. Agriculture Census reports in units of farms, which may contain one or more flocks. To calculate the number of flocks in each size strata, farm and flock were equated for each stratum with less than 100,000 hens per farm. For farms with more than 100,000 hens, an average capacity of 110,000 hens per flock was used.

Table A-1. Four strata of flock size used in production module.					
Strata & Number of hens per flock (range)	Number of flocks in each stratum				
10,000-19,999	1892				
20,000-49,999	1134				
50,000-99,999	519				
≥ 100,000	1483				
Total	5028				

#### C. Production Module Variables

## 1. Prevalence of SE-positive flocks

#### a. Evidence

The prevalence of SE-positive flocks (which produce SE-positive eggs) is used to determine the number of SE-positive flocks in the production module.

Prevalence surveys conducted in 1991 (Ebel et al.,1992) and 1995 (Hogue et al. 1997) allow development of the estimate of the national prevalence of SE-positive flocks. In 1991, 111 (27%) of 406 flocks sampled were SE-positive. In 1995, 136 (45%) of 305 flocks sampled were SE-positive.

The prevalence of SE-positive flocks for the above studies was determined through slaughter surveys of spent hens. Spent hens are hens which no longer lay eggs at a rate which is commercially viable, and these hens are considered 'spent'. Spent hens are removed from production; typically via slaughter. These slaughter surveys of spent hens used a two-stage sampling design whereby flocks were initially selected and then intestinal tract samples from 300 hens in each flock were collected and cultured for SE.

Because of seasonal effects in the results, data from the 1995 spent hen survey conducted by Hogue et al. (1997) and data from the 1991 national spent hen survey by Ebel et al. (1992) are combined in the estimation of national prevalence of SE-positive flocks. We assume that combining both surveys may more accurately reflect an average prevalence over a full year. Therefore, our analysis begins with an assumption that 247 (i.e., 111 + 136) of 711 (406 + 305) flocks sampled in the spent hen surveys were SE-positive.

The 1991 and 1995 national spent hen surveys evaluated prevalence of SE-positive flocks at the regional level. Regions were not necessarily sampled in proportion to the actual number of flocks resident in each region (Table 2). As Table A-2 shows, 310 (44%) of 711 flocks were sampled in the Northern region in the 1991 and 1995 surveys, yet only 27% of the U.S. flock resides in the Northern region. To use the regional spent hen data to estimate a national prevalence of SE-positive flocks, the regional results must be weighted by the proportion of the national flock in each region. These weights are calculated on the basis of the 1992 Agriculture Census and are equal to 27%, 33%, 26%, and 14% for the Northern, Southeastern, Central, and Western regions, respectively.

Table A-2. Combined 1991 and 1995 spent hen survey results by U.S. regions<sup>1</sup> and percent of U.S. flock in each region<sup>2</sup>.

U.S. Regions	Number of flocks sampled	Number of flocks SE-positive (%) in survey	Percent of U.S. flock located in region
Northern	310	163 (52%)	27
Southeastern	92	7 (8%)	33
Central	232	59 (25%)	26
Western	77	18 (23%)	14
Total	711	247 (35%)	100

- 1. Adapted from Hogue et al. (1997).
- 2. Adapted from 1992 Agriculture Census.

For each region in Table A-2, the prevalence of SE-positive flocks is estimated as a Beta function with parameters of s+1 and n-s+1 - Beta(s+1, n-s+1), where s is the number of positive flocks and n is the total number of flocks sampled in each region. Regional prevalence is multiplied by the proportion of US flocks in the region to determine the contribution of each region to the national prevalence of SE-positive flocks. The sum of these calculations is the apparent national prevalence of SE-positive flocks. Apparent prevalence is an epidemiologic term which refers to prevalence calculated without adjustments for the sensitivity of surveillance tests used (Martin et al., 1987). In our case, the mean apparent prevalence is calculated as 28%.

The national prevalence estimate using the spent hen survey data is further adjusted to account for imperfect surveillance sensitivity. Higher prevalence flocks are more likely to be detected than lower prevalence flocks. Within-flock prevalence results from the combined 1991 and 1995 national spent hen surveys are shown in Table A-3. The most frequent within-flock prevalence detected in these surveys was 0.33%. Surveillance sensitivity [p (detection | truly positive flock)] is calculated as  $1 - (1-\text{wprev})^n$  where 'wprev' is the within-flock prevalence of 0.33% , the surveillance sensitivity of 300 samples is 63%. This result means that if 100 flocks of hens which all have a within-flock prevalence level of 0.33% were tested according to the spent hen protocol (i.e. 300 samples from each flock), then 37 flocks (100 flocks  $\times$  (1-0.63) = 37) would be incorrectly classified as negative flocks.

To estimate the surveillance sensitivity of the spent hen surveys, a model based on the within-flock prevalence results shown in Table A-3 was developed. This model showed that the sample of 300 hens per flock used in the spent hen surveys had a sensitivity of 76%. This finding means that 76% of truly positive flocks were correctly classified as SE-positive in these surveys, while 24% of truly positive flocks were incorrectly classified as SE-negative. This estimate of sensitivity had a standard deviation of 2.5%.

The true national SE-flock prevalence is calculated by dividing the apparent national prevalence by 0.76.

Because the spent hen surveys did not identify flocks by their sizes, SE prevalence within each size stratum may actually vary over a considerable range. At one extreme, all flocks fitting within a certain stratum may have been SE-positive in these surveys. At the other extreme, none of the flocks in a stratum may have been positive, or even sampled. Therefore, an algorithm was developed to allow prevalence to range from 0% to 100% within a stratum while maintaining a constant national prevalence. This adjustment serves to increase the uncertainty in estimates of SE flock prevalence.

Table A-3. Within-flock prevalence levels based on 1991 and

1995 spent hen survey results.

1995 spent hen survey results.							
Number of positive samples	Number of flocks	Freq	Within-flock prevalence <sup>1</sup>				
1	77	31.2%	0.33%				
2	39	15.8%	0.67%				
3	23	9.3%	1.00%				
4	18	7.3%	1.33%				
5	9	3.6%	1.67%				
6	6	2.4%	2.00%				
7	8	3.2%	2.33%				
8	7	2.8%	2.67%				
9	8	3.2%	3.00%				
10	4	1.6%	3.33%				
11	6	2.4%	3.67%				
12	4	1.6%	4.00%				
13	4	1.6%	4.33%				
14	2	0.8%	4.67%				
15	2	0.8%	5.00%				
16	6	2.4%	5.33%				
17	1	0.4%	5.67%				
18	3	1.2%	6.00%				
19	3	1.2%	6.33%				
21	2	0.8%	7.00%				
22	3	1.2%	7.33%				
23	1	0.4%	7.67%				
24	1	0.4%	8.00%				
25	1	0.4%	8.33%				
26	2	0.8%	8.67%				
27	2	0.8%	9.00%				
28	1	0.4%	9.33%				
36	1	0.4%	12.00%				
39	1	0.4%	13.00%				
42	1	0.4%	14.00%				
44	1	0.4%	14.67%				

<sup>&</sup>lt;sup>1</sup> Within-flock prevalence calculated as number of positive samples divided by 300 hens sampled.

b. Value of the variable:

On average, we estimate 37% of egg-laying flocks are SE-positive.

c. Distribution: Beta(267,443)

A Beta distribution is used to estimate the proportion of a population that is positive when positive/negative sampling data is available. The formula for this estimate is Beta (s+1, n-1+1) where s is the number of positive samples and n is the total number of samples collected (Vose, 1996)

The following histogram shows the distribution of the percent of egg-laying flocks which are SE-positive flocks. This graph reflects our uncertainty regarding true prevalence. It shows that we estimate with 90% confidence that the prevalence of SE positive flocks lies between 32% (5<sup>th</sup> percentile) and 43% (95<sup>th</sup> percentile).

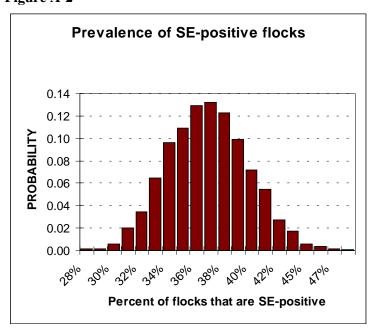
Mean 37%

Standard 3%
deviation

5<sup>th</sup> 32%
percentile

95<sup>th</sup> 43%

Figure A-2



## 2. Frequency of high prevalence flocks

#### a. Evidence

As explained earlier, a high prevalence flock is a flock which is SE-positive and produces SE-positive eggs at a higher rate than the average rate for all SE-positive flocks. The attribute (or factor) of prevalence among SE-positive flocks has been dichotomized in this module into high and low levels - i.e., high prevalence SE-positive flocks produce SE-positive eggs at high rates and low prevalence SE-positive flocks produce SE-positive eggs at low rates. This dichotomy is probably a simplification, as it is possible that a continuum from low to high prevalence SE-positive flocks exists in the population of all SE-positive flocks. It is advantageous to explicitly model at least two levels of SE-positive flocks based on SE-positive egg frequency so that the relative contribution of these different levels to the total number of SE-positive eggs produced per year can be evaluated.

The frequency of high prevalence SE-positive flocks is a conditional probability which predicts the proportion of flocks that are in the high prevalence category given that the flocks are SE-positive.

There is experimental and field evidence which supports the concept of variable expression of SE infection in flocks. Several researchers have experimentally demonstrated that there are differences between SE strains which make some strains of SE more capable of causing more severe manifestations of infection than others - e.g., higher rates of SE-positive eggs - (Gast et al., 1990; Gast et al., 1992; Guard-Petter et al., 1993, 1995, 1996, 1997a, 1997b; Gast et al., 1995; Gast et al., 1996: Thiagarajun et al., 1994). Other experimental research has shown that there exist host factors that might influence the severity of SEinfection (Lindell et al., 1994; Tellez et al., 1994; Qin et al., 1995; Manning et al., 1994; Phillips et al., 1995; Bumstead et al., 1993). Unfortunately, very little research is available concerning flock management or environmental risk factors which might explain increased severity of SE infection in flocks. Studies by Henzler (1992, 1998) have suggested that rodents play a role in the epidemiology of SE in the environment of egg-laying flocks. Poor control of rodent populations may allow greater transmission of SE into and throughout the flock in a layer house with consequent higher prevalence rates of SE-positive hens. Mallinson (1997) has suggested that environmental moisture levels are associated with Salmonella prevalence in manure drag swabs and on broiler (i.e. chicken) carcasses. The relationship of the prevalence of SE-positive hens within a flock and environmental factors such as temperature, ventilation, stocking density, caging, and feeding/watering systems are less well studied in the literature.

A study by Schlosser et al. (1995) provides one source of field based evidence regarding high prevalence flocks. In that study, 43 SE-positive flocks were

investigated as part of the Pennsylvania Pilot Project. Eight of the 43 flocks were characterized as having the highest rates of SE-positive eggs cultured from them. These flocks were also SE-positive in >50% of the environmental samples collected from them. Because of this apparent correlation between environmental and egg sampling, the designation of a SE-positive flock as a 'high prevalence flock' can be made based on either type of sampling. In this same study, 27 of these 43 SE-positive flocks had no SE-positive eggs - i.e. no SE was cultured from any of the eggs tested. Such findings provide strong evidence for low prevalence flocks.

In another field study of SE-positive flocks, Henzler et al. (1994) showed that the greatest rate of SE-positive eggs were found in two of four flocks investigated. In both of these high prevalence flocks, >50% of environmental samples were SE-positive.

Combining the data from Schlosser et al. (1995) and Henzler et al. (1994) implies that of 47 SE-positive flocks intensively investigated, 10 (21%) could be characterized as high prevalence flocks. However, just as with the national prevalence estimate, we must adjust this percentage by the surveillance sensitivity of testing used to determine these flocks' SE-positive status. In these two studies, environmental samples were collected and cultured. It is reported that environmental sampling is equivalent to sampling 50 hens' caeca from a flock at slaughter (Kingston, 1981). Therefore, environmental sampling is less sensitive at detecting positive flocks than the spent hen surveys (which sampled 300 hens).

A consequence of this lower surveillance sensitivity is that if we determined that 21% of all SE-positive flocks were high prevalence, we would be wrong. Based on a sample size of 50 hens, we calculated the sensitivity of environmental testing as 49%, using the same methodology as explained for the national prevalence adjustment. This means that 51% of SE-positive flocks would be incorrectly classified as negative (i.e., false negative) using environmental testing. Clearly, the SE-positive flocks incorrectly classified would not include those characterized as high prevalence flocks, since these flocks - by definition - are easily detected as positive using environmental testing. Therefore, the false negative flocks must be included in those flocks considered low prevalence. Consequently, we must reduce the frequency of high prevalence flocks calculated from Schlosser et al. (1995) and Henzler et al. (1994) by approximately one-half (i.e., 21% x 49% = 11%).

## b. Value of the variable:

On average, we estimate that 11% of SE-positive flocks are high prevalence flocks.

## c. Distribution: Beta(11,85)

The following histogram shows the distribution of the percent of SE-positive flocks that are high prevalence flocks. This graph reflects our uncertainty of the fraction of positive flocks that are high prevalence flocks. It can be seen from this graph that we are 90% confident that the true value for this variable lies between 6% (5<sup>th</sup> percentile) and 17% (95<sup>th</sup> percentile).

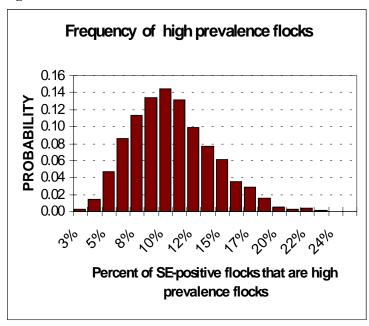
Mean 11%

Standard 3% deviation

5<sup>th</sup> 6% percentile

95<sup>th</sup> 17% percentile

Figure A-3



### 3. Frequency of SE-positive molted flocks

#### a. Evidence

Molting is a process used in commercial egg flocks to extend the period of egg production by rejuvenating the reproductive systems of hens. Flocks that are not molted typically only lay eggs for one year. Molting is the shedding of feathers followed by growth of new feathers. Layers that molt also cease egg production during the molt period. In commercial flocks, molting is induced by the restriction of light and feed. This process provides the necessary stimulus to urge the hens in a flock to undergo a molt. This period usually averages about 4 weeks during which the flock is essentially non-productive. Once molting is complete the birds are stimulated - primarily by light - to begin laying again. Although slightly fewer eggs are produced after molt, these eggs tend to be larger than eggs produced before molt.

There is epidemiologic evidence which associates molting with higher prevalence of SE in flocks. Molted SE-positive flocks also seem to produce SE-positive eggs more frequently than their non-molted counterparts. Experimentally, Holt et al. (1996,1995,1994,1993,1992) have demonstrated that molting is associated with increased numbers of SE in hens' intestinal tracts, and higher rates of SE-positive eggs are produced following molt. Schlosser et al. (1995) demonstrated similar results in a field study during the Pennsylvania Pilot Project. In that study, molted flocks produced SE-positive eggs twice as frequently as non-molted flocks for a period up to 140 days following molt.

The frequency distribution for SE-positive molted flocks is derived from statistics reported by the USDA - National Agricultural Statistics Service (USDA-NASS). These statistics state that approximately 22% of flocks in egg production - at any time of the year - are flocks that have molted.

## b. Value of the variable:

On average, we estimate that 22% of flocks producing eggs on any given day are flocks that were previously molted.

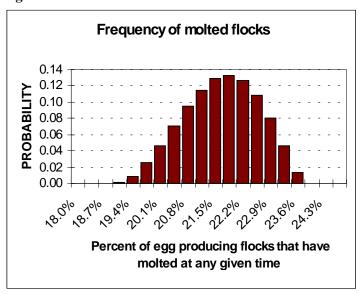
## c. Distribution: Pert(19%,22%,24%)

The following histogram shows the distribution for the frequency of molted flocks variable. This graph reflects our uncertainty regarding the fraction of SE-positive flocks that were previously molted. It can be seen from this graph that we are 90% confident that the true value for this variable lies between 20% (5<sup>th</sup> percentile) and 23% (95<sup>th</sup> percentile).

Mean 22%

Standard 0.9%
deviation 5th 20%
percentile 95th 23%
percentile

Figure A-4



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4. Number of days of increased frequency of SE-positive eggs post-molt

## a. Evidence

SE-positive flocks that are molted do not perpetually produce SE-positive eggs more frequently than flocks that are not molted. Instead, there appears to be a period immediately after molt when these flocks are at higher risk of producing more positive eggs.

The frequency distribution for 'number of days of increased frequency of SE-positive eggs post-molt' is based on the data from the Pennsylvania Pilot Project (Schlosser et al, 1995). In that study, SE-positive molted flocks were sampled from 0 to 20 weeks (140 days) post-molt. During this time period, these molted flocks produced nearly twice as many SE-positive eggs as similarly studied SE-positive flocks that were not molted.

## b. Value of the variable:

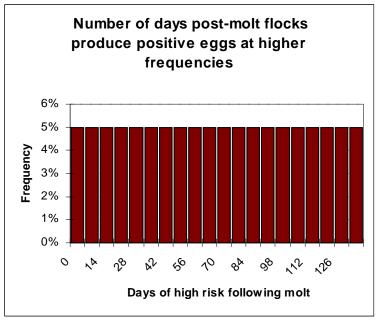
On average, we estimate that SE-positive flocks will produce more positive eggs during the first 70 days following molt.

c. Distribution: Uniform(0,140 days)

A uniform distribution is used to model variables for which only a minimum and maximum value are available. The available evidence shows that flocks were between 0 (minimum) and 20 weeks (maximum) post-molt when they produced eggs at higher frequencies

The following histogram shows the frequency distribution for the number of days post-molt when SE-positive eggs are produced more frequently. This graph reflects the variability in this value for individual SE-positive molted flocks. It can be seen from this graph that 90% of such flocks will experience between 7 days (5<sup>th</sup> percentile) and 133 days (95<sup>th</sup> percentile) of increased SE-positive eggs following their molt.

Figure A-5



Mean 70
Standard 40
deviation 7
percentile 95th 133
percentile

Page 43

## 5. Eggs per flock per day

#### a. Evidence

To estimate eggs produced per flock per day, we begin with the 1992 U.S. Agriculture Census data used to determine the number of flocks by size strata (Table A-1 on page 31). For each stratum, the average number of hens per flock is calculated as the total number of commercial laying hens, divided by the number of flocks, in the stratum. For example, the 1992 U.S. Agriculture Census reports the 10,000-19,000 flock size stratum comprised 21.7 million hens and 1892 flocks. Therefore, the average flock in this stratum consisted of 11,470 hens (21.7 million / 1892). Similar calculations for the 20-49K, 50-99K, and >100K flock size strata resulted in average flock sizes of 27,222, 68,691, and 110,00 hens per flock, respectively. The average flock size across all four strata is 50,154 hens per flock.

We assume the average hen in a flock produces 0.72 eggs per day (i.e., she will produce 72 eggs during a 100 day period). This average daily egg production was based on a published egg production curve (Rahn, 1977), which was adjusted for improved egg production - using annual USDA-NASS statistics - since that curve was derived.

#### b. Value of the variable

:

The model assumes there are 5028 flocks in the U.S. (Table 1, page 2). The average flock size is 50,154 hens per flock. Each hen produces 72 eggs per 100 days. Therefore, the average flock produces 13 million eggs per year. Furthermore, the model predicts an average of 65 billion eggs produced per year (i.e., 5028 flocks x 50,154 hens/flock x 0.72 eggs/day x 365 days). This annual production estimate is consistent with 1995-1996 statistics published by USDANASS.

c. Distribution: Eggs produced per day is a constant in the model.

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6. Frequency of SE-positive eggs in high prevalence, SE-positive / not molted flocks

#### a. Evidence

As was discussed earlier, SE-positive flocks which are molted appear to produce more SE-positive eggs. For this reason, a distinction is made between SE-positive flocks which are molted and SE-positive flocks which are not molted.

Egg culturing results from the eight flocks identified as high prevalence, SE-positive flocks in the Pennsylvania Pilot Project (Schlosser et al., 1995) are incorporated into this estimate (see pp. 37). Of 113,000 eggs collected from these flocks, 56 were found SE-positive. These flocks had the greatest average SE-positive egg frequency of the 43 flocks uniformly studied. The ages of flocks in this cohort ranged from 20 weeks to 72 weeks old. None of these flocks were molted.

Egg sampling results from two flocks identified through traceback procedures from human SE outbreaks showed that 41 of 15,980 eggs collected were SE-positive (Henzler, et al., 1994). These two flocks demonstrated particularly high rates of SE-positive eggs and SE-positive environmental samples, and for this reason meet the definition of high prevalence, SE-positive flocks.

Egg culture results from 8 flocks in California which were SE-positive with phage type 4 variety of SE (Kinde et al., 1996) are similar to the other results for high prevalence, SE-positive / not molted flocks. Of 85,360 eggs collected from these flocks, 58 eggs were SE-positive.

From egg sampling completed in these three studies, we find that a total of 214,340 eggs have been sampled from flocks we would characterize as high prevalence/not molted. From these samples, 155 eggs were found SE-positive. Therefore, these results suggest that high prevalence/not molted flocks produce 7 SE-positive eggs in every 10,000 eggs they lay (i.e., 155/214,340 = 0.07%). This rate is equivalent to 1 SE-positive egg in every 1383 eggs produced, or 2 SE-positive eggs in every 2766 eggs produced. We use the latter estimate to form our distribution for this variable.

#### b. Value of the variable:

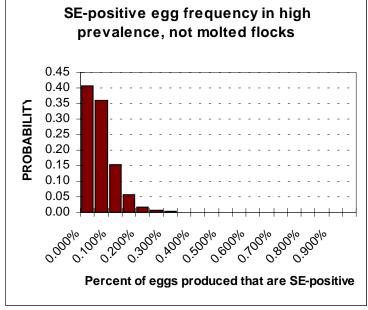
On average, we estimate that high prevalence / not molted flocks will produce 7 SE-positive eggs in every 10,000 eggs they produce.

c. Distribution: Gamma(2,(2766)<sup>-1</sup>)

The gamma distribution is used to estimate low frequency events (Vose, 1996). The formula for this estimate is gamma (s, 1/n), where s is the number of positive samples and n is the total number of samples collected. Because the egg sampling results we used were from just a few flocks, we wanted to model our estimate of positive egg frequency with the greatest degree of uncertainty possible based on the available evidence. Setting s equal to 2 and proportionally adjusting n provides the greatest variance for a gamma distribution variable.

The following histogram shows the distribution for the frequency of SE-positive eggs in high prevalence / not molted flocks. This graph reflects our uncertainty regarding the fraction of eggs produced by high prevalence / not molted flocks that are SE-positive. It can be seen from this graph that we are 90% confident that this frequency is between 1 SE-positive egg in every 10,000 eggs produced (5<sup>th</sup> percentile) and 17 SE-positive eggs in every 10,000 produced (95<sup>th</sup> percentile). These percentile values are equivalent to 0.01% (5<sup>th</sup> percentile) and 0.17% (95<sup>th</sup> percentile).

Figure A-6



Mean 0.07%

Standard 0.05% deviation

5<sup>th</sup> 0.01% percentile

95<sup>th</sup> 0.17% percentile

7. Frequency of SE-positive eggs in high prevalence, SE-positive / molted flocks

#### a. Evidence

The frequency at which SE-positive eggs are produced by high prevalence, SE-positive / molted flocks is used to calculate the number of SE-positive eggs produced by high prevalence, SE-positive flocks during the high risk period following molt (as explained on pp. 42). For flocks in this category, the rate of SE-positive eggs during other times of the egg production year is modeled in the same way as for high prevalence, SE-positive / not molted flocks.

The distribution for the frequency of SE-positive eggs in high prevalence, SE-positive / molted flocks is calculated using analysis of the data from the Pennsylvania Pilot Project (Schlosser et al., 1995). In that analysis, the aggregate frequency of SE-positive eggs was compared for flocks that were in egg production during the 20-week time frame before molting and for flocks that were in egg production during the 20-week time frame after molting. This approach controlled for confounding variables in the data due to the effect of the age of the hen. Based on these findings, it appears that the average effect of molting is to double the frequency of SE-positive eggs in SE-positive flocks after the molting process.

<u>Data Source</u>		<u>s</u>	<u>n</u>	Number of SE-positive eggs per 10,000 eggs
Cablesses et al. 1005	Post-molt (0-20 wks)	31	74,000	4.2
Schlosser et al., 1995	Pre-molt (0-20 wks)	14	67,000	2.1

s =the number of SE-positive eggs

 $\label{eq:new_section} n = \text{the number of eggs sampled in high prevalence, SE-positive} \, / \, \, \text{molted or not} \\ \text{molted flocks}$ 

The frequency of SE-positive eggs in high prevalence, SE-positive / molted flocks was modeled by adjusting the frequency calculated for high prevalence, SE-positive / not molted flocks. The adjustment is based on a risk ratio developed from the data of the Pennsylvania Pilot Project. The risk ratio associated with molting equals the frequency of SE-positive eggs detected in post-molt flocks, divided by the frequency of SE-positive eggs detected in premolt flocks.

Multiplying the positive egg frequency for high prevalence / not molted flocks (i.e., 0.07%) by the relative risk associated with molting (i.e., 2) equals 0.14%. Therefore, applying the effect of molting to high prevalence flocks implies that these flocks produce 14 SE-positive eggs per 10,000 eggs they lay during the high risk period following molt. Alternatively, this estimate equals 1 SE-positive egg in every 714 eggs produced, or 2 SE-positive eggs in every 1429 eggs produced. We use this latter estimate to form our distribution for this variable.

#### b. Value of the variable:

On average, we estimate that high prevalence / molted flocks produce 14 (0.14%) SE-positive eggs in every 10,000 eggs they lay during the high risk postmolt period (which averages about 70 days). To validate this estimate, we analyzed data from 13 SE-positive flocks that were molted during the Pennsylvania Pilot Project. There were four flocks with above average frequencies of SE-positive eggs which we classified as high prevalence / molted. The remaining nine flocks were classified as low prevalence / molted. We found that 76 (0.167%) of 46,000 eggs cultured from the four high prevalence flocks were SE-positive for a rate of 16.7 SE-positive eggs per 10,000 eggs. This result closely compares to the mean frequency of SE-positive eggs (0.14%) or 14 SE-positive eggs per 10,000 eggs) which was calculated using the methods outlined above.

## c. Distribution: $Gamma(2,(1429)^{-1})$

The following histogram shows the distribution for the frequency of SE-positive eggs in high prevalence / molted flocks. This graph reflects our uncertainty regarding the fraction of eggs produced by high prevalence / molted flocks that are SE-positive. It can be seen from this graph that we are 90% confident that this frequency is between 2 SE-positive eggs in every 10,000 eggs produced (5<sup>th</sup> percentile) and 41 SE-positive eggs in every 10,000 produced (95<sup>th</sup> percentile). These percentile values are equivalent to 0.02% (5<sup>th</sup> percentile) and 0.41% (95<sup>th</sup> percentile).

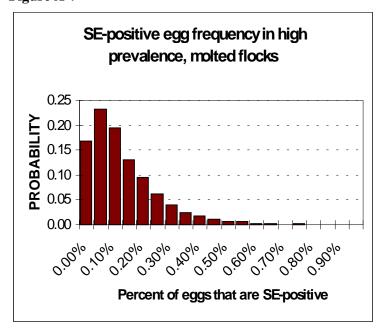
Mean 0.16%

Standard 0.13% deviation

5<sup>th</sup> 0.02% percentile

95<sup>th</sup> 0.41% percentile

Figure A-7



8. Frequency of SE-positive eggs in low prevalence, SE-positive / not molted flocks

#### a. Evidence

Just as the available evidence supports the existence of high prevalence, SE-positive flocks, such evidence also supports the concept that a greater proportion of SE-positive flocks are low prevalence, SE-positive flocks.

The frequency of SE-positive eggs in low prevalence, SE-positive / not molted flocks is calculated using the following data:

Data Sources	S	n	Frequency of SE-positive eggs per 10,000 eggs
Schlosser et al., 1995	22	381,000	0.6
Henzler et al, 1994	2	10,140	2.0

s = the number of SE-positive eggs

**n** = the number of eggs sampled in SE-positive, low prevalence/not molted flocks.

The data from 43 unmolted flocks in the Pennsylvania Pilot Project cited previously (Schlosser et al., 1995) was used to determine the frequency of SE-positive eggs in low prevalence, SE-positive flocks. Those SE-positive flocks not classified as high prevalence (n=8) in that study were classified as low prevalence (n=35), and the aggregate egg culture results from the low prevalence, SE-positive flocks are represented in the table above. In a study by Henzler et al., (1994) of four flocks, two flocks showed low frequencies of SE-positive egg cultures and SE-positive environmental samples. Combining the results of these two studies, we find that 24 SE-positive eggs were detected in 391,140 eggs sampled from low prevalence / not molted flocks.

Therefore, these results suggest that low prevalence / not molted flocks produce 6 SE-positive eggs in every 100,000 eggs they lay. However, this estimate only applies to flocks that were found SE-positive using environmental testing since flocks in these studies were detected using environmental sampling. Because within-flock prevalence levels in low prevalence, SE-positive flocks can be very low, it is not reasonable to apply a frequency of SE-positive eggs which is derived from data collected in flocks found to be SE-positive on the basis of environmental testing. We expect there are SE-positive flocks that would not be detected using environmental testing (i.e., false negative flocks), but would be producing SE-positive eggs. However, these false negative flocks would not be expected to produce SE-positive eggs at a lower frequency than flocks whose level of infection was sufficient to be detected via environmental sampling.

In the national spent hen surveys, nearly 50% of the SE-positive flocks detected had within-flock prevalence levels between 0.33% and 0.66% (Table A-2, page 32). Within-flock prevalence measures the proportion of hens that have SE in

their intestinal tract. However, infected hens typically produce SE-positive eggs only during the first week of their four week infection (Leslie, 1996). In other words, one-quarter of infected hens at any given time are capable of producing SE positive eggs. Furthermore, it is estimated that a positive hen in her first week of infection only produces SE-positive eggs 8% of the time during that week (Leslie, 1996).

A within-flock prevalence of 0.33% means that 33 hens are SE-positive in a flock of 10,000 hens. One-quarter of these hens - or eight hens in a flock of 10,000 - are assumed to be in their first week of infection. These eight hens will produce 3 SE-positive eggs in one week (8 hens x 7 days x 0.72 eggs/day x 8% SE-positive eggs). The flock of 10,000 hens will produce a total of 50,400 eggs in a week (10,000 hens x 7 days x 0.72 eggs/day). Therefore, we estimate that an SE-positive egg frequency of approximately 0.005% (i.e. 3 / 50,400)) corresponds to a within-flock prevalence of 0.33%. For a within-flock prevalence of 0.66%, the corresponding positive egg frequency is 0.0096% (i.e. 9.6 SE-positive eggs per 100,000 eggs). These frequencies of SE-positive eggs closely agree with the mean frequency of SE-positive eggs (0.006% or 6 SEpositive eggs per 100,000 eggs produced) developed from the data in the table above. Therefore, these findings support using a frequency of 6 SE-positive eggs per 100,000 eggs produced in flocks detected via environmental testing or the spent hen survey methods. However, these findings also support the need to develop another frequency distribution for SE-positive eggs which applies to flocks whose within-flock prevalence levels are below the detection threshold of the spent hen surveys.

Low prevalence, SE-positive flocks are further subdivided into two categories. Category 1 low prevalence, SE-positive flocks are those low prevalence, SE-positive flocks found to be SE-positive through the national spent hen surveys. Category 2 low prevalence, SE-positive flocks are those low prevalence, SE-positive flocks which were not detected by the spent hen surveys.

To determine the frequency of SE-positive eggs for Category 2 low prevalence flocks, the within-flock prevalence levels of SE-positive hens for flocks not detected to be SE-positive flocks by the spent hen surveys must first be determined. Bayes theorem is used to calculate P( prev | test-), which is a mathematical expression which states 'the probability of the within-flock prevalence of SE-positive hens given that the flocks tested SE-negative in the spent hen surveys'. Bayes theorem states;

$$P(\text{prev} | \text{test} - ) = P(\text{test} - | \text{prev}) * P(\text{prev}) / \sum [P(\text{test} - | \text{prev}) * P(\text{prev})]$$

The probability of a flock testing SE-negative given different within-flock prevalence levels - i.e. P( test - | prev) - was calculated by determining the probability of no SE-positive results in a sample of 300 hens when a flock had a within-flock prevalence which ranged from 0.001% to 1% (i.e. 1 SE-positive hen per 100,000 hens to 1 SE-positive hen per 100 hens). The probability of

different within-flock prevalence levels - i.e. P(prev) - was determined by fitting a distribution to the national spent hen data. From our calculations of P(prev | test - ), we developed a distribution for SE-positive eggs by assuming that one-fourth of positive hens in a flock were at risk of producing SE-positive eggs, and these at-risk hens produced positive eggs 8% of the time (i.e., as we estimated above). This analysis estimated that the average Category 2 low prevalence / not molted flock produced 5 SE-positive eggs in every 1 million eggs they laid.

#### b. Value of the variable:

- (1) Frequency of SE-positive eggs in Category 1 low prevalence, SE-positive / not molted flocks.
  - On average, we estimate that Category 1 low prevalence / not molted flocks produce 6 SE-positive eggs in every 100,000 eggs they lay.
- (2) Frequency of SE-positive eggs in Category 2 low prevalence, SE-positive / not molted flocks.

On average, we estimate that Category 2 low prevalence / not molted flocks produce 5 SE-positive eggs in every 1 million eggs they lay.

#### c. Distribution:

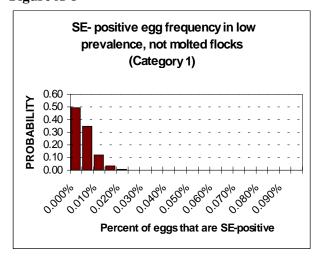
(1) Category 1:  $Gamma(2,(33,333)^{-1})$ 

The following histogram shows the distribution for the frequency of SE-positive eggs in Category 1 low prevalence / not molted flocks. This graph reflects our uncertainty regarding the fraction of eggs produced by Category 1 low prevalence / not molted flocks that are SE-positive. It can be seen from this graph that we are 90% confident that this frequency is between 1 SE-positive egg in every 100,000 eggs produced (5<sup>th</sup> percentile) and 14 SE-positive eggs in every 100,000 produced (95<sup>th</sup> percentile). These percentile values are equivalent to 0.001% (5<sup>th</sup> percentile) and 0.014% (95<sup>th</sup> percentile).

Mean 0.006%

Standard 0.004%
deviation 5th 0.001%
percentile 95th 0.014%
percentile

Figure A-8

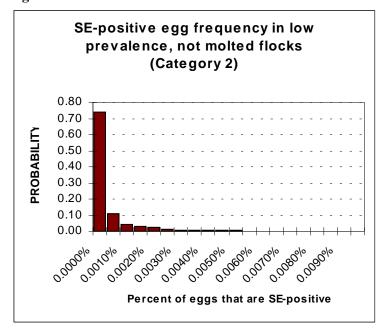


(2) Category 2: Cumulative(0%,0.06%,0.00001%::0.01%,0.04::0.99)

This distribution is defined such that the minimum value is zero, and the maximum value is 6 per 10,000 (0.06%). Furthermore, the distribution is based on a range of values extending from 1 in 10 million (0.00001%) to 1 in 10,000 (0.01%), which occur with cumulative probabilities beginning with 0.04 and ending with 0.99. These cumulative probabilities ensure that extreme values (i.e., maximum and minimum) only occur about 5% of the time.

The following histogram shows the distribution for the frequency of SE-positive eggs in Category 2 low prevalence / not molted flocks. This graph reflects our uncertainty regarding the fraction of eggs produced by Category 2 low prevalence / not molted flocks that are SE-positive. It can be seen from this graph that we are 90% confident that this frequency is between 1 SE-positive egg in every 1 million eggs produced (5<sup>th</sup> percentile) and 2 SE-positive eggs in every 100,000 produced (95<sup>th</sup> percentile). These percentile values are equivalent to 0.0001% (5<sup>th</sup> percentile) and 0.002% (95<sup>th</sup> percentile).

Figure A-9



 Mean
 0.0005%

 Standard deviation
 0.001%

 5th percentile
 0.0001%

 95th percentile
 0.002%

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- 9. Frequency of SE-positive eggs in low prevalence, SE-positive / molted flocks
  - a. Evidence

The frequency at which SE-positive eggs are produced by low prevalence, SE-positive / molted flocks is used to calculate the number of SE-positive eggs produced by low prevalence, SE-positive flocks during the time period following the molting process of the hens when the rate of SE-positive eggs increases. For flocks in this category, the frequency of SE-positive eggs during the remainder of the year, when the flock is not molting, is modeled in the same way as for low prevalence, SE-positive / not molted flocks.

The frequency of SE-positive eggs in low prevalence, SE-positive / molted flocks is calculated by adjusting the frequency of SE-positive eggs of low prevalence, SE-positive / not molted flocks. This method uses the same data for molted flocks as was presented for the frequency of SE-positive egg from high prevalence, SE-positive / molted flocks (pp. 48).

#### Value of the variable:

(1) Frequency of SE-positive eggs in Category 1 low prevalence, SE-positive / molted flocks.

On average, we estimate Category 1 low prevalence / molted flocks produce 1.3 SE-positive eggs in every 10,000 eggs they lay. This frequency is essentially twice the frequency we estimated for Category 1 low prevalence / not molted flocks.

Analysis of the data from the 13 molted flocks in the Pennsylvania Pilot Project validates our estimate of the frequency of SE-positive eggs for low prevalence, SE-positive / molted flocks. By classifying the nine SE-positive flocks with below-average frequencies of SE-positive eggs as low prevalence, SE-positive flocks, it was found that 16 eggs (0.019%) of 83,000 eggs cultured from this cohort of nine flocks were SE-positive eggs. This result closely compares to the mean positive egg frequency (0.013%) calculated for Category 1 - low prevalence, SE-positive / molted flocks.

(2) Frequency of SE-positive eggs in Category 2 low prevalence, SE-positive / molted flocks.

On average, we estimate that Category 2 low prevalence / molted flocks produce 1 SE-positive egg in every 100,000 eggs they lay. Again, this is essentially double the rate estimated for Category 2 flocks that were not molted.

- b. Distribution of the variable:
  - (1) Category 1:  $Gamma(2,(16,500)^{-1})$

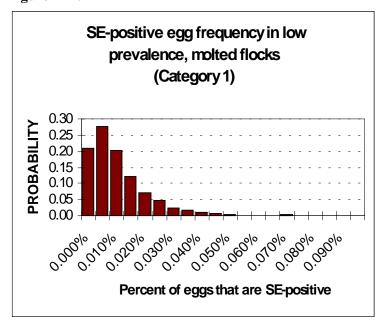
The following histogram shows the distribution for the frequency of SE-positive eggs in Category 1 low prevalence / molted flocks. This graph reflects our uncertainty regarding the fraction of eggs produced by Category 1 low prevalence / molted flocks that are SE-positive. It can be seen from this graph that we are 90% confident that this frequency is between 1 SE-positive egg in every 100,000 eggs produced (5<sup>th</sup> percentile) and 3 SE-positive egg in every 10,000 produced (95<sup>th</sup> percentile). These percentile values are equivalent to 0.001% (5<sup>th</sup> percentile) and 0.03% (95<sup>th</sup> percentile).

Mean	0.013%
Standard deviation	0.01%
5 <sup>th</sup> percentile	0.001%
95 <sup>th</sup> percentile	0.03%

Maan

0.0120/

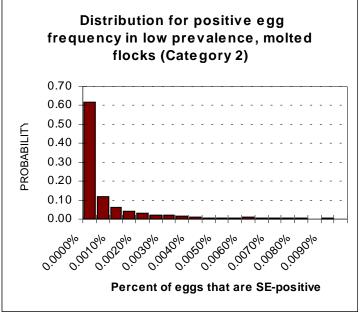
Figure A-10



(2) Category 2: Cumulative distribution for Category 2 not molted flocks times the relative risk of molting (approximately 2).

> The following histogram shows the distribution for the frequency of SEpositive eggs in Category 2 low prevalence / molted flocks. This graph reflects our uncertainty regarding the fraction of eggs produced by Category 2 low prevalence / molted flocks that are SE-positive. It can be seen from this graph that we are 90% confident that this frequency is between 1 SE-positive egg in every 1 million eggs produced (5<sup>th</sup> percentile) and 5 SE-positive eggs in every 100,000 produced (95th percentile). These percentile values are equivalent to 0.0001% (5<sup>th</sup> percentile) and 0.005% (95th percentile).

Figure A-11



Mean 0.001% Standard 0.002% deviation 0.0001% percentile 95<sup>th</sup> 0.005% percentile

Page 58

### 10. Destination of SE-positive eggs post-production

The physical distribution of SE-positive eggs to plants for further processing as shell eggs or into liquid egg products after egg production determines the total number SE-positive eggs which will be modeled in the Shell Egg Processing/Distribution module and the Egg Products Processing/Distribution module. These modules - Shell Egg Processing/Distribution module and Egg Products Processing/Distribution module - actually simulate the growth of SE in a single egg.

The likelihood that any egg is distributed to the shell egg processor or to the egg products processor is based on USDA-NASS and Agricultural Marketing Service (AMS) statistics. Every year 76.1% of all eggs are marketed through shell egg processing, while 23.9% of all eggs are marketed through egg products. After processing and grading, 5% of all shell eggs are determined to be restricted eggs because these eggs have checks (i.e. cracks in the shell detected during candling of the egg) in the shell or are dirty, and these restricted eggs are diverted to plants which make egg products. However, 10% of restricted eggs are determined to be inedible, and these inedible eggs are either destroyed or labeled and handled as either for animal food or for industrial use.

We calculate the number of SE-positive eggs per year that are destined for egg products and shell egg markets based on the following percentages.

Shell eggs	72.3%
Egg Products	23.9%
Shell eggs diverted to egg products	3.4%
Inedible eggs	0.4%
	=====
	100%

## **D.** Output of Production Module

The output of the production module consists of;

- 1) the number of SE-positive eggs produced annually by commercial U.S. layer flocks (which are divided into four different types of flocks),
- 2) the percent of SE-positive eggs in all eggs produced annually by these four different types of flocks, and
- 3) the number of SE-positive eggs distributed to the processors of shell eggs for distribution and to the processors of egg products for distribution.

To calculate these outputs, the module first calculates the number of SE-positive flocks, then categorizes these SE-positive flocks by prevalence (i.e., high or low) and molting status. For each of the four types of flocks, the module calculates the annual number of eggs produced, then applies the appropriate frequency of SE-positive eggs for the type of flock in order to calculate the number of SE-positive eggs produced per annum by each flock type and by all flock types together. The module outputs also include the number of SE-positive flocks, the number of high prevalence SE-positive flocks, the number of high prevalence SE-positive eggs produced per year by high prevalence SE-positive / molted flocks, etc.

To calculate total eggs produced by one of the four types of SE-positive flocks (e.g., high prevalence, SE-positive / not molted flock), the daily egg production of each of the four types of SE-positive flocks is multiplied by the number of days each type of flock is in production. This product is then multiplied by the number of flocks of each type. This calculation is done for each size stratum within each of the four types of SE-positive flock.

The number of SE-positive eggs produced by each of the four types of SE-positive flocks is calculated as Normal( $n\lambda$ ,  $(\lambda n)^{1/2}$ ) distribution, where n is the number of eggs produced per year by a specific SE-positive flock type, and  $\lambda$  is the frequency of SE-positive eggs of each type of flock. The Central Limit Theorem states that the sum of a group of random variables will have a Normal( $n\mu$ , $\sigma n^{1/2}$ ) distribution, where n is the number of random variables in the group and  $\mu$  and  $\sigma$  are the mean and standard deviation of the variables. In this case, n is the number of eggs produced per year and each egg has some likelihood of being an SE-positive egg based on the frequency of SE-positive eggs associated with the type of flock that produced the egg.

From the total number of SE-positive eggs, the number of SE-positive eggs sent to shell egg processors/distributors and to egg products processors/distributors is calculated. Also calculated are the number of SE-positive eggs initially sent to shell egg processing/distribution, but later diverted to egg products processing/distribution. Finally, the number of SE-positive eggs which are treated as inedible is calculated. Inedible eggs are not considered further in the model.

The following histogram depicts the distribution for the total number of SE-positive eggs produced per year, as predicted by the Production module. On average, we estimate that about

3.3 million SE-positive eggs are produced from the 65 billion eggs laid per year. It can be seen from this graph that we are 90% confident that the total number of SE-positive eggs produced per year is between 974,303 (5<sup>th</sup> percentile) and 7,386,495 (95<sup>th</sup> percentile).

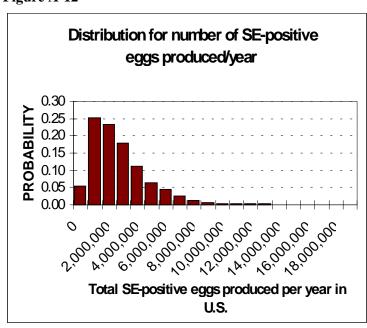
 Mean
 3,312,064

 Standard deviation
 2,164,706

 5th percentile
 974,303

 95th percentile
 7,386,495

Figure A-12



The following histogram depicts the distribution for the overall frequency of SE-positive eggs produced per year in the U.S. Overall frequency of SE-positive eggs is calculated by dividing the total number of SE-positive eggs produced per year (on average, 3.3 million) by the total number of eggs produced per year (on average, 65 billion). We estimate there is an average of 5 SE-positive eggs produced per 100,000 eggs laid by U.S. hens. It can be seen from this histogram that we are 90% confident that the frequency of SE-positive eggs produced per year is between 1 SE-positive egg per 100,000 eggs produced (5<sup>th</sup> percentile) and 11 SE-positive eggs per 100,000 eggs produced (95<sup>th</sup> percentile). These percentile values are equivalent to 0.001% (5<sup>th</sup> percentile) and 0.011% (95<sup>th</sup> percentile).

Figure A-13

	Distribution for frequency of SE- positive eggs in all eggs produced/year
PROBABILITY	0.20 0.15 0.10 0.05 0.00
	Percent SE-positive eggs in all eggs produ per year in U.S.

 Mean
 0.005%

 Standard deviation
 0.003%

 5th percentile
 0.001%

 95th percentile
 0.011%

Table A-4. Mean percentage contribution to total SE-positive eggs by type of SE-positive flocks and by stratum within a type of flock							
Type of Flock				20-49K			

Type of Flock		Total	10-19K	20-49K	50-99K	>100K
			hens per	hens per	hens per	hens per
			Flock	Flock	Flock	Flock
High prevalence, SE-+ / molted		15.44%	1.28%	1.79%	2.26%	10.12%
High prevalence, SE-+ / not molted		50.94%	4.43%	6.34%	7.15%	33.02%
Low prevalence, SE-+ / molted	Cat. 1	7.49%	0.64%	0.91%	1.05%	4.88%
	Cat. 2	0.25%	0.02%	0.03%	0.04%	0.17%
Low prevalence, SE-+ / not molted	Cat. 1	25.02%	2.15%	3.03%	3.52%	16.31%
	Cat. 2	0.85%	0.07%	0.10%	0.12%	0.55%
		100%				
	•	•		•		

The results depicted in Table A-4 above demonstrate the relative contribution of each of the four types of SE-positive flocks to the total number of SE-positive eggs produced per year in the production module. The percentage attributed to each of the four types of SE-positive flocks by the production module is located in the column labeled 'Total'. Note that two of the types of flocks are each further subdivided into two categories, as discussed earlier in the text. The contribution of each of the four types of SE-positive flocks to the total production of SE-positive eggs based on the size of a flock (i.e. stratum) is shown in the four columns on the right hand side of Table A-4.

High prevalence, SE-positive / not molted flocks produce a slight majority of the positive eggs (50.9%). On average, 11% of SE-positive flocks are high prevalence flocks. Furthermore, an average of 22% of these high prevalence flocks are molted. Therefore, only 9% of all SE-positive flocks are high prevalence / not molted flocks. Yet, these flocks are estimated to produced over one-half the SE-positive eggs per year.

On average, high prevalence flocks are responsible for about two-thirds of all positive eggs (i.e. 15.44% + 50.94%). Low prevalence flocks account for the remaining one-third. However, Category 2 low prevalence flocks' contribution to total SE-positive eggs per year is minimal. Molted flocks contribute roughly one-quarter of all positive eggs (i.e 15.44% + 7.49%). This proportion is essentially the same as the proportion of SE positive flocks that are molted. By flock size strata, the largest stratum (i.e.  $\geq 100,000$  hens per flock) contributes almost two-thirds of the positive eggs (i.e. 10% + 33% + 4.8% + 16%), while the remaining one-third of positive eggs are distributed among the other three strata in nearly equivalent proportions.

Table A-5. Number of SE-positive eggs per year distributed to shell egg and egg products markets.

	Number of SE-positive eggs/year				
Marketing option	Mean 5 <sup>th</sup> 95 <sup>th</sup> percentile percent				
Direct to shell egg processing	2,242,526	704,373	5,340,066		
Diverted from shell egg market to egg products	106,225	33,365	252,950		
Inedible eggs	11,803	3,707	28,106		
Direct to egg products processing	741,356 232,859 1,765,37				

Table A-5 demonstrates the distribution of SE-positive eggs to shell egg processing and egg products processing predicted by the Production module. On average, we estimate that 2.2 million SE-positive eggs per year are processed and distributed as shell eggs. Furthermore, we estimate that an average of 847,581 SE-positive eggs (741,356 + 106,225) per year are processed and distributed as egg products. We estimate that about 11,803 SE-positive eggs are disposed of as inedible before further processing. For all eggs produced per year (65 billion), 47 billion are processed and distributed as shell eggs, 18 billion are processed and distributed as egg products, and 0.26 billion are inedible.

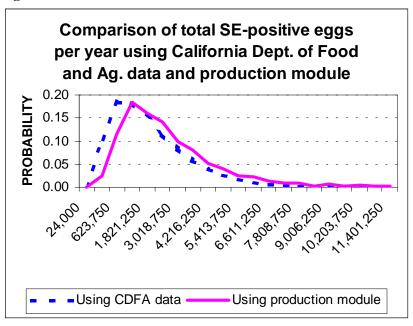
#### E. Module Validation

A comparison of the output distribution of SE-positive eggs from the production module with a distribution of SE-positive eggs developed from survey data provided by the California Department of Food and Agriculture (CDFA) (Ian Gardner, personal communication) shows good correspondence. A random survey conducted in California found 1 SE-positive pooled egg sample in 1416 pooled egg samples, where 20 eggs were pooled per sample. This result is equivalent to finding 1 SE-positive egg in 28,320 eggs. Applying our methodology to this data results in a Gamma (2,(14,160)<sup>-1</sup>) distribution with a mean of 0.35 SE-positive eggs per 10,000 eggs. Because eggs in this survey were sampled without knowledge of the SE status of the flock, this distribution is applicable to all eggs produced per year.

To compare the survey findings of the CDFA to the production module's predictions, the national prevalence of SE-positive flocks used in the production module was adjusted to only reflect the results of the national spent hen surveys from the Western region. Because the production module is designed to model national levels, this adjustment is necessary to compare California's data to the production module results. California is the largest egg producing state in the U.S.. Therefore, data generated from that state is reasonably expected to reflect the Western U.S.. For the sake of simplicity, the prevalence of SE-positive flocks in the western region was applied to all U.S. flocks, and the distribution for total number of SE-positive eggs produced per year is demonstrated. Figure A-14 compares the two distributions (one based on the data from CDFA and one based on the production module). Figure A-14 shows that the output of the production module is very similar to the distribution of SE-positive eggs independently derived from the CDFA data. This CDFA data was not used in the development

of the production module. Therefore, this comparison of the CDFA data and the production module output is a demonstration of this module's validity. The actual numbers of SE-positive eggs predicted by the production module are not relevant to this discussion. Instead, the intention of the Figure A-14 is to demonstrate the robustness of the production module. Such agreement is reassuring. The mean frequencies of SE-positive eggs among all eggs produced for the California data and the production module are 0.35 per 10,000 eggs and 0.5 per 10,000 eggs, respectively. These frequencies predict the mean numbers of SE-positive eggs per year of 2.2 million SE-positive eggs from the California validation output and 2.7 million SE-positive eggs from the output of the production module.

Figure A-14



# F. Sensitivity Analysis

A sensitivity analysis of the production module was completed to determine the degree to which the input variables were correlated with this module's output. When an input variable is highly correlated with the output variable, it is expected that adjustments in the input distribution will result in substantial changes in the output distribution and may be a control point for intervention.

This sensitivity analysis examined the degree to which input variables were correlated with the total number of SE-positive eggs produced per year in the U.S. All input variables were evaluated. Specifically, these input variables included;

- ♦ Prevalence of SE-positive flocks
- ♦ Frequency of high prevalence flocks
- ♦ Frequency of molted flocks
- ♦ Days of high risk post-molt
- Positive egg frequency variables for high/low prevalence, molted/not molted flocks.

Our sensitivity analysis of the production module indicates the following variables are most correlated with the number of SE-positive eggs produced per year (Table 6):

- positive egg frequency in unmolted high and low prevalence flocks.
- frequency of SE-positive flocks (especially in the largest flocks),
- frequency of high prevalence flocks.

Because high prevalence flocks contribute a disproportionately large number of positive eggs to the total SE-positive eggs per year (as demonstrated in Table A-4, see page 63), it is not surprising that variables that serve to estimate the role of high prevalence flocks should be correlated with the output of the production module. For example, when the module predicts a lower than average percent of flocks are high prevalence flocks, the total number of SE-positive eggs per year calculated by the module is reduced. Similarly, when the module predicts a lower than average frequency of SE-positive eggs in high prevalence flocks, total positive eggs per year declines.

Because molted flocks only contribute a proportional number of positive eggs to the total SE-positive eggs per year (Table A-4, see page 63), the variables associated with molting are not correlated with the output of the production module. Such results are somewhat surprising given the much higher frequencies at which molted flocks produce SE-positive eggs. However, these flocks experience high positive egg frequencies for a limited time - typically 70 days in the module. Furthermore, molted flocks do not produce any eggs for an average of 30 days because

they cease production during molt. Consequently, when the frequency of molted flocks, or the positive egg frequencies in molted flocks, is varied in the module, there is less effect on the predicted total number of SE-positive eggs per year than other variables.

Strategies to mitigate the likelihood of SE-positive eggs at the production level include mechanisms to prevent the entry of SE into commercial flocks, remove SE from the poultry house environment, and reduce within-flock transmission. Effects of these strategies can be incorporated into the production module through the variables listed above. For example, mechanisms to prevent entry of SE into flocks include the testing of replacement pullets before the introduction of replacement pullets into the layer house, using SE-free feedstuffs, and other biosecurity practices. These mechanisms could be modeled through their reduction in the frequency of SE-positive flocks. Similarly, the effect of improved rodent control on reducing an important reservoir of SE in poultry environments could be modeled as a reduction in frequency of SE-positive flocks. Improved rodent control might also reduce within-flock transmission of SE, thereby causing a reduction in the frequency of high prevalence flocks.

Table A-6. Correlation between input variables and SE-positive eggs produced/year.	
Name of input variable	Correlation Coefficient
Positive egg frequency in high prevalence, SE-positive / not molted flocks	0.62
Prevalence of SE-positive flocks in >100K hens per flock size stratum	0.54
Positive egg frequency in low prevalence, SE-positive / not molted flocks - Cat. 1	0.34
Frequency of high prevalence, SE-positive flocks	0.26
Apparent SE-positive flock prevalence in >100K flock size stratum	0.24
Apparent SE-positive flock prevalence calculated from spent hen surveys	0.10
Sensitivity of environmental testing	0.09
Days of high risk post-molt	0.08
SE-positive egg frequency in high prevalence, SE-positive / molted flocks	0.06
Apparent within-flock prevalence for test-negative flocks	0.03

### **G.** Production Module Limitations

The limitations of the production module stem from the data used to estimate its variables and the scope of the risk assessment.

Data used to estimate the frequency of high/low prevalence flocks was generated from a large industry-government project conducted in Pennsylvania (Schlosser et al., 1995). Although this data is extremely valuable because of its uniqueness (i.e., it is the only such data published concerning U.S. commercial flocks), it can only be expected to have direct relevance to the Pennsylvania industry. Similar limitations also apply for the positive egg frequency data generated by this project.

Another limitation is the lack of temporal data to provide information regarding patterns of flock prevalence and within-flock prevalence over time. Estimates of positive egg frequencies in Category 2 low prevalence flocks, for example, were developed based on theoretically static within-flock prevalence levels because empiric evidence was lacking. Furthermore, estimates of positive eggs were restricted to an annual basis because monthly or quarterly estimates could not be supported by the available data.

The scope of this risk assessment also applied limitations for the production module. This assessment was designed to model the number of human illnesses resulting from internally SE-positive eggs, then evaluate the effectiveness of various mitigations in reducing these illnesses. Therefore, we developed a baseline model which reflects the status quo regarding SE occurrence. The model does not attempt to reflect changes in SE occurrence over time.

In Europe, the emergence of a particularly virulent strain of SE has resulted in a persistent and pervasive SE problem. While the current situation in the U.S. is not as severe as Europe's, research by Guard-Petter (1997) suggests that the U.S. situation may yet evolve to the level of the European experience. Specifically, Guard-Petter et al. (1997) argue that SE populations on farms can undergo differentiation and result in growth to higher cell densities, expression of virulence factors, and overall higher penetrance within flocks. Such developments could result in greater frequencies of high prevalence flocks in the U.S. over time. The recent detection of Phage type 4 SE - the SE type currently affecting Europe - in U.S. commercial egg flocks (Kinde et al., 1996 and Hogue et al., 1997) suggests the need for heightened surveillance for SE in this country. However, this assessment does not currently address a hypothetically increasing prevalence of severe SE infections in the U.S. Nevertheless, as more epidemiologic data becomes available, the model can be adapted to evaluate such changes.

### H. Mathematics of the Production Module

## 1. Definitions of Constants

 $N_i = \text{number of flocks in size stratum I, (I=1,2,3,4)}$ 

 $B_i = \text{number of hens per flock in stratum I, (I=1,2,3,4)}$ 

E =frequency of eggs per hen per day (0.72)

 $Z_1$  = percent of eggs only marketed to the shell egg market per year

 $Z_2$  = percent of eggs marketed to the shell egg market then diverted to egg products market per year

 $Z_3$  = percent of eggs marketed directly to the egg products market per year

 $Z_4$  = percent of eggs that are inedible per year

### 2. Variables

## a. Input variables

 $p_i = prevalence (percent) of SE-positive flocks in stratum.$ 

h = frequency (percent) of high prevalence flocks among SE-positive flocks

m = frequency (percent) of flocks that have molted

 $f_j$  = frequency (percent) of SE-positive eggs produced by SE-positive flocks in category j, (j=1,2,3,4,5,6)

k = number of days a flock is out of production due to molting

d ≡ number of days post-molt that molted flocks experience increased positive egg frequencies

s = percent of SE-positive flocks detected when sampling 300 hens per flock

### 3. Calculations

## a. <u>Intermediate variables</u>

### (1) Number of positive flocks

HPM₁ = number of SE-positive, high prevalence, molted flocks in stratum I

$$HPM_i = N_i * p_i * h * m$$

HPUM<sub>i</sub> = number of SE-positive, high prevalence, unmolted flocks in stratum I

$$HPUM = N_i * p_i * h * (1-m)$$

LPM1; ≡ number of Category 1, SE-positive, low prevalence, molted flocks in stratum I

$$LPM1_i = N_i * p_i * (1-h) * s * m$$

LPM2; = number of Category 2, SE-positive, low prevalence, molted flocks in stratum I

$$LPM2_i = N_i * p_i * (1-h) * (1-s) * m$$

LPUM1; ≡ number of Category 1, SE-positive, low prevalence, unmolted flocks in stratum I

$$LPUM1_i = N_i * p_i * (1-h) * s * (1-m)$$

LPUM2; ≡ number of Category 2, SE-positive, low prevalence, unmolted flocks in stratum I

$$LPUM2_i = N_i * p_i * (1-h) * (1-s) * (1-m)$$

# (2) Total eggs per year produced by positive flocks

 $EHPM_i = total eggs produced per year by <math>HPM_i$  flocks

$$EHPM_{i} = HPM_{i} * B_{i} * E * (365 days - k)$$

DEHPM<sub>i</sub> = total eggs produced by HPM<sub>i</sub> flocks during d days following molt,

$$DEHPM_i = Normal(HPM_i * B_i * E * \mu_d, (HPM_i * B_i * E * \sigma_d)^{0.5})$$

YEHPM; = total eggs produced by HPM; flocks during the rest of the year,

$$YEHPM_i = EHPM_i - DEHPM_i$$

EHPUM<sub>i</sub> ≡ total eggs produced per year by HPUMi flocks

$$EHPUM_i = HPUM_i * B_i * E * 365 days$$

ELPM1<sub>i</sub> ≡ total eggs produced per year by LPM1<sub>i</sub> flocks

$$ELPM1_{i} = LPM1_{i} * B_{i} * E * (365 days - k)$$

DELPM1; = total eggs produced by LPM1; flocks during d days following molt,

DELPM1<sub>i</sub>=Normal(LPM1<sub>i</sub> \* B<sub>i</sub> \* E \* 
$$\mu_d$$
, (LPM1<sub>i</sub> \* B<sub>i</sub> \* E \*  $\sigma_d$ )<sup>0.5</sup>)

YELPM1; ≡ total eggs produced by LPM1; flocks during the rest of the year,

$$YELPM1_i = ELPM1_i - DELPM1_i$$

ELPM2; ≡ total eggs produced per year by LPM2; flocks

$$ELPM2_{i} = LPM2_{i} * B_{i} * E * (365 days - k)$$

DELPM2<sub>i</sub> = total eggs produced by LPM2<sub>i</sub> flocks during d days following molt,

$$DELPM2_{i} = Normal(LPM2_{i} * B_{i} * E * \mu_{d}, (LPM2_{i} * B_{i} * E * \sigma_{d})^{0.5})$$

YELPM2; ≡ total eggs produced by LPM2; flocks during the rest of the year,

$$YELPM2_i = ELPM2_i - DELPM2_i$$

ELPUM1; ≡ total eggs produced per year by LPUM1; flocks

$$ELPUM1_i = LPUM1_i * B_i * E * 365 days$$

ELPUM2<sub>i</sub> ≡ total eggs produced per year by LPUM2<sub>i</sub> flocks

$$ELPUM2_i = LPUM2_i * B_i * E * 365 days$$

# (3) SE-positive eggs per year produced by positive flocks

SEHPM<sub>i</sub> = SE-positive eggs produced per year by HPMi flocks

$$SEHPM_i =$$

$$Normal[(YEHPM_i * f_1), (YEHPM_i * f_1)^{0.5}] + Normal[(DEHPM_i * f_2), (DEHPM_i * f_2)^{0.5}]$$

where  $f_1$  is the positive egg frequency for high prevalence, unmolted flocks and  $f_2$  is the positive egg frequency for high prevalence, molted flocks.

SEHPUM<sub>i</sub>- SE-positive eggs produced per year by HPUMi flocks

$$SEHPUM_i = Normal[(EHPUM_i * f_1), (EHPUM_i * f_1)^{0.5}]$$

SELPM1; = SE-positive eggs produced per year by LPM1; flocks

$$SELPM1_i =$$

$$Normal[(YELPM1_1 * f_3), (YELPM1_1 * f_3)^{0.5}] + Normal[(DELPM1_1 * f_4), (DELPM1_1 * f_4)^{0.5}]$$

where  $f_3$  is the positive egg frequency for Category 1 low prevalence, unmolted flocks and  $f_4$  is the positive egg frequency for Category 1 low prevalence, molted flocks.

SELPM2<sub>i</sub> ≡ SE-positive eggs produced per year by LPM2<sub>i</sub> flocks

$$SELPM2_{i} =$$

$$Normal[(YELPM2_i * f_5), (YELPM2_i * f_5)^{0.5}] + Normal[(DELPM2_i * f_6), (DELPM2_i * f_6)^{0.5}]$$

where  $f_5$  is the positive egg frequency for Category 2 low prevalence, unmolted flocks and  $f_6$  is the positive egg frequency for Category 2 low prevalence, molted flocks.

SELPUM1<sub>i</sub> ≡ SE-positive eggs produced per year by LPUM1<sub>i</sub> flocks

$$SELPUM1_i = Normal[(ELPUM1_i * f_3), (ELPUM1_i * f_3)^{0.5}]$$

SELPUM2<sub>i</sub> ≡ total eggs produced per year by LPUM2<sub>i</sub> flocks

$$SELPUM2_i = Normal[(ELPUM2_i * f_5), (ELPUM2_i * f_5)^{0.5}]$$

b. Output variables

Y = total number of SE-positive eggs produced per year

$$Y = \sum_{i} [SEHPM_{i} + SEHPUM_{i} + SELPM1_{i} + SELPM2_{i} + SELPUM1_{i} + SELPUM2_{i}]$$

 $Y_1$  = total number of SE-positive eggs marketed to shell eggs per year

$$\mathbf{Y}_1 = \mathbf{Y} * \mathbf{Z}_1$$

 $Y_2$  = total number of SE-positive eggs marketed as egg products per year

$$Y_2 = Y * (Z_2 + Z_3)$$

 $Y_3 = \text{total number of SE-positive eggs that are inedible per year}$ 

$$Y_3 = Y * Z_4$$

### I. References

Bumstead, N. and Barrow, P., 1993. Resistance to *Salmonella gallinarum*, S. pullorum, and S. enteritidis in inbred lines of chickens. Avian Diseases 37:638-647.

Ebel, E.D., David, M.J., and Mason, J., 1992. Occurrence of *Salmonella enteritidis* in the U.S. commercial egg industry: report on a national spent hen survey. Avian Diseases 36:646-654.

Gast, R.K., and Benson, S.T., 1996. Intestinal colonization and organ invasion in chicks experimentally infected with *Salmonella enteritidis* phage type 4 and other phage types isolated from poultry in the United States. Avian Diseases 40: 853-857.

Gast, R.K. and Benson, S.T., 1995. The comparative virulence for chicks of *Salmonella enteritidis* phage type 4 isolates and isolates of phage types commonly found in poultry in the United States. Avian Diseases 39: 567-574.

Gast, R.K. and Beard, C.W., 1992. Evaluation of a Chick Mortality Model for Predicting the Consequences of *Salmonella enteritidis* Infections in Laying Hens. Poultry Science 71: 281-287.

Gast, R.K. and Beard, C.W., 1990. Isolation of *Salmonella enteritidis* from internal organs of experimentally infected hens. Avian Diseases 34: 991-993.

Guard-Petter, J., 1997. Induction of flagellation and a novel agar-penetrating flagellar structure in *Salmonella enterica* grown on solid media: Possible consequences for serological identification. FEMS Microbiological Letters 149: 173-180.

Guard-Petter, J., 1993. Detection of Two Smooth Colony Phenotypes in A *Salmonella enteritidis* Isolate Which Vary in Their Ability To Contaminate Eggs. Applied and Environmental Microbiology 59: 2884-2890.

Guard-Petter, J., Henzler, D.J., Rahman, M.M., and Carlson, R.W., 1997. On-farm monitoring of mouse-invasive *Salmonella enterica* serovar Enteritidis and a model for its association with the production of contaminated eggs. Applied and Environmental Microbiology 63: 1588-1593.

Guard-Petter, J., Keller, L.H., Rahman, M.M., Carlson, R.W., and Silvers, S., 1996. A novel relationship between O-antigen variation, matrix formation, and invasiveness of *Salmonella enteritidis*. Epidemiology and Infection 117: 219-231.

Guard-Petter, J., Lakshmi, B., Carlson, R., and Ingram, K., 1995. Characterization of lipopolysaccharide heterogeneity in *Salmonella enteritidis* by an improved gel electrophoresis method. Applied and Environmental Microbiology 51: 2845-2851.

Henzler, D.J., Ebel, E., and Sanders, J., 1994. *Salmonella* Enteritidis in Eggs from Commercial Chicken Layer Flocks Implicated in Human Outbreaks. Avian Diseases 38: 37-43.

Henzler, D.J., Kradel, D.C., and Sischo, W.M., 1998. The chicken layer environment and isolation of *Salmonella enteritidis* from eggs. American Journal of Veterinary Research, accepted for publication.

Henzler, D.J. and Opitz, H.M., 1992. The role of mice in the epizootiology of *Salmonella enteritidis* infection on chicken layer farms. Avian diseases 36: 625-631.

Hogue et al., 1997. Surveys of *Salmonella* Enteritidis in unpasteurized liquid egg and spent hens at slaughter. Journal of Food Protection. In press.

Holt, P., 1996. Infection of stressed chickens by airborne *Salmonella enteritidis*. Proceedings of the American Society for Microbiology Meetings.

Holt, P.S., 1995. Horizontal transmission of *Salmonella enteritidis* in molted and unmolted laying chickens. Avian Diseases 39: 239-249.

Holt, P.S., Buhr, R.J., Cunningham, D.L., and Porter Jr., R.E., 1994. Effect of Two Different Molting Procedures on a *Salmonella enteritidis* Infection. Poultry Science 73: 1267-1275.

Holt, P.S., and Porter, Jr., R.E., 1993. Effect of Induced Molting on the Recurrence of a Previous *Salmonella enteritidis* Infection. Poultry Science 72: 2069-2078.

Holt, P.S., and Porter, Jr., R.E., 1992. Effect of induced molting on the course of infection and transmission of *Salmonella enteritidis* in white leghorn hens of different ages. Poultry Science 71: 1842-1848.

Humphrey, T.J., Williams, A., McAlpine, K. Lever, Guard-Petter, J., and Cox, J.M., 1996. Isolates of *Salmonella enterica* Enteritidis PT4 with enhanced heat and acid tolerance are more virulent in mice and more invasive in chickens. Epidemiology and Infection 117: 79-88.

Humphrey, T.J., Chart, H., Baskerville, A., and Rowe, B., 1991. The influence of age on the response of SPF hens to infection with *Salmonella enteritidis* PT4. Epidemiology and Infection 106: 33-43.

Humphrey, T.J., Whitehead, A., Gawler, A.H.L., Henley, A., and Rowe, B., 1991. Numbers of *Salmonella enteritidis* in the contents of naturally contaminated hens' eggs. Epidemiology and Infection 106: 489-496.

Humphrey, T.J., Baskerville, A., Mawer, S., Rowe, B., and Hopper, S., 1989. *Salmonella enteritidis* phage type 4 from the contents of intact eggs: A study involving naturally infected hens. Epidemiology and Infection 103: 415-423.

Kinde, H., Read, D.H., and Gardner, I.A., 1996. *Salmonella enteritidis*, phage type 4 infection in a commercial layer flock in southern California: Bacteriologic and epidemiologic findings. Avian Diseases 40: 665-671.

Kingston, D.J., 1981. A comparison of culturing drag swabs and litter for identification of infections with *Salmonella* spp. in commercial chicken flocks. Avian Dis. 25:513-516.

Leslie, J., 1996. Simulation of the transmission of *Salmonella enteritidis* phage type 4 in a flock of laying hens. Veterinary Record 139:388-391.

Lindell, K.A., Saeed, A.M., and McCabe, A.M., 1994. Evaluation of Resistance of Four Strains of Commercial Laying Hens to Experimental Infection with *Salmonella enteritidis* Phage Type Eight. Poultry Science 73: 757-762.

Mallinson, E.T., Joseph, S.W., Carr, L.E., and Wabeck, C.J., 1997. Litter management is critical to food safety, performance. Feedstuffs May 19, 47-52.

Manning, J.G., Hargis, B.M., and Hinton Jr., A., 1994. Effect of selected antibiotics and anticoccidials on *Salmonella enteritidis* cecal colonization and organ invasion in leghorn chicks. Avian diseases 38: 256-261.

Martin, S.W., Meek, A.H., Willeberg, P., 1987. Veterinary Epidemiology Principles and Methods. Iowa State University Press.

Phillips, R.A., and Opitz, H.M., 1995. Pathogenicity and persistence of *Salmonella enteritidis* and egg contamination in normal and Infectious Bursal Disease Virus-infected leghorn chicks. Avian Diseases 39:778-787.

Qin, Z.R., Arakawa, A., Baba, E., Fukata, T., Miyamoto, T., Sasai, K. and Withanage, G.S.K., 1995. Eimeria tenella infection induces recrudescence of previous *Salmonella enteritidis* infection in chickens. Poultry Science 74: 1786-1792.

Rahman, M.M., Guard-Petter, J. and Carlson, R.W., 1997. A virulent isolate of *Salmonella enteritidis* produces a *Salmonella* typhi-like lipopolysaccharide. Journal of Bacteriology 179: 2126-2131.

Rahn, A.P., 1977. A strategic planning model for commercial laying flocks. Poultry Science 56:1579-1584.

Schlosser, W., Henzler, D., Mason, J., Hurd, S., Trock, S., Sischo, W., Kradel, D., and Hogue, A., 1995. *Salmonella enteritidis* Pilot Project Progress Report. Washington, DC: U.S. Government Printing.

Tellez, G., Dean, C.E., Corrier, D.E., DeLoach, J.R., Jaeger, L., and Hargis, B.M., 1994. Effect of dietary lactose on cecal morphology, pH, organic acids, and *Salmonella enteritidis* organ invasion in Leghorn chicks. Poultry Science 73: 636-642.

Thiagarajun, D., Saeed, A.M., and Asem, E.K., 1994. Mechanism of transovarian transmission of *Salmonella enteritidis* in laying hens. Poultry Science 73: 89-98.

Van de Giessen, A.W., Ament, A.J.H.A., and Notermans, S.H., 1994. Intervention strategies for *Salmonella enteritidis* in poultry flocks: a basic approach. International Journal of Food Microbiology 21: 145-154.

Vose, D., 1996. Quantitative Risk Analysis: A guide to Monte Carlo Simulation Modeling. John Wiley and Sons, Ltd.

Waltman, W.D., Horne, A.M., Pirkle, C., and Johnson, D.C., 1992. Prevalence of *Salmonella enteritidis* in Spent Hens. Avian Diseases 36: 251-255.

Winkler, R.L., 1972. An introduction to Bayesian inference and decision. Holt, Rinehart, and Winston, Inc.